pointes. We studied the effects of paroxetine on human *ether-a-go-go*-related gene (hERG) channels expressed in *Xenopus* oocytes and on action potential in guinea pig ventricular myocytes. The hERG encodes the pore-forming subunits of the rapidly-activating delayed rectifier K^+ channel (I_{kr}) in the heart. Mutations in hERG reduce I_{kr} and cause type 2 long QT syndrome (LQT2), a disorder that predisposes individuals to life-threatening arrhythmias. Paroxetine induced concentration-dependent decreases in the current amplitude at the end of the voltage steps and hERG tail currents. The inhibition was concentration-dependent and time-dependent, but voltage-independent during each voltage pulse. The S6 domain mutation Y652A did not affect the drug-induced hERG current block. In guinea-pig ventricular myocytes held at 36°C, treatment with 0.4 μM paroxetine for 5 min decreased the action potential duration at 90% of repolarization (APD_{90}) by 4.3%. Our results suggest that paroxetine is a blocker of the hERG channels, providing a molecular mechanism for the arrhythmogenic side effects during the clinical administration of paroxetine.

3070-Pos Board B175

Quinidine Block of Shab K Channels: Irreversible Collapse of the K^+ conductance, and Characterization of an External Selectivity Filter K^+ binding Site

Froylan Gomez-Lagunas, Elisa Carrillo.

Quinidine is a commonly used antiarrhythmic agent and a useful tool to study ion channels. We will show that: (1) quinidine (Qd) equilibrates within seconds across the plasma membrane of Sf9 insect cells, blocking the open pore of Shab K channels from the intracellular side of the membrane in a voltage-dependent manner with 1:1 stoichiometry. (2) On binding to the channels Qd interacts with pore K^+ ions in a mutually destabilizing manner. As a result, (3) when the channels are blocked by Qd with the cell bathed in an external medium lacking K^+, the Shab conductance G_K collapses irreversibly, despite the presence of a physiological [K^+] in the intracellular solution. (4) The Qd-promoted collapse of Shab G_K resembles the collapse of Shaker G_K observed with 0 K^+ solutions on both sides of the membrane: thus the extent of G_K drop depends on the number of activating pulses applied in the presence of Qd, but it is independent of the pulse duration. Taken together the observations indicate that, as in Shaker, the Qd-promoted collapse of Shab G_K occurs during deactivation of the channels, at the end of each activating pulse, with a probability of 0.1 per pulse at −80 mV. (5) Finally, we will compare the Ki (inhibition constant) with which different external cations destabilize the binding of Qd against the potency with which the same cations inhibit the collapse of G_K, in an attempt to characterize both the selectivity of the external K^+ binding sites (s1/s2) and their role in the stability of G_K.

3071-Pos Board B176

Effect of MTS Reagents on Wildtype and *h*Kv1.3_V417C Mutant Channels and its Implications for C-Type Inactivation

Sonja I. Schmid, Stephan Grissmer.

The voltage-gated *h*Kv1.3 channel, member of the *Shaker*-related potassium channels, is involved in T-cell activation and is characterized by its typical C-type inactivation. To characterize the three-dimensional structure of the C-type inactivated state a cysteine was introduced at position 417 (*Shaker* position 467) in the *h*Kv1.3 channel and a putative involvement in C-type inactivation was determined using MTS-reagents. MTSEA application led, in contrast to wildtype channels, to a fast and irreversible current reduction through *h*Kv1.3_V417C channels in the open or inactivated state indicating that a modification of both states was possible. This modification could be prevented by verapamil. In contrast, the closed state of this mutant channel could not be modified by MTSEA. Furthermore a current reduction was observed only when the positively charged MTSET was applied intracellularly and not when applied extracellulary to *h*Kv1.3_V417C channels. These experiments indicated that the binding site for MTS-reagents is intracellular and that a modification of the cysteine at position 417 in the *h*Kv1.3_V417C mutant channel was possible in the open and also inactivated state of the channel. In addition, the fact that the inactivated state of the *h*Kv1.3_V417C mutant channel could be modified by MTSEA indicated also that the activation gate must be open during inactivation, the side chain of the cysteine at position 417 does not move during inactivation in a way that it is not available for modification any more and the channel is, using the model by Cuello et al. (2010, *Nature* **466**:203), in the open-inactivated state.

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3072-Pos Board B177

Discrimination Among Heteromeric Potassium Channels by Pore-Blocking Conotoxins

Rocio K. Finol-Urdaneta, Stefan Becker, Baldomero M. Olivera, Heinrich Terlau, Robert J. French.

Screening of specificity of drugs affecting K channels commonly uses homotetrameric channels assembled following expression of a single monomer. However, in many tissues, voltage-gated K currents may reflect the properties of heteromeric channels. Recently, we described a cardioprotective action of the Kv1.2-blocking conopeptide κM-RIIIK, but concluded that this was unlikely to result from an interaction with homomeric Kv1.2 channels (Chen et al., 2010, J.Biol.Chem. 285:4882). Here, we examine target discrimination, among heteromers, of the related conotoxins κM-RIIIJ and κM-RIIIK by testing their activity on 12 different Kv1.2-containing channels, each formed after expression of a single dimeric construct. Expression of homodimeric Kv1.2 yielded channels with toxin sensitivity similar to homotetramers, suggesting that dimerization, *per se*, does not affect toxin sensitivity. κM-RIIIK was most potent against Kv1.2 homotetramers and 1.2/1.7 heteromeric channels, but did not discriminate based on the order of connectivity in the latter. κM-RIIIJ was most potent against 1.1/1.2 constructs, without regard for connectivity, but showed significant discrimination based on connectivity between the two constructs for both 1.5/1.2 and 1.6/1.2 heteromers. Preliminary data for two Kunitz family conopeptides Conkunitzin-S1 and Conkunitzin-S2 suggest that each of these peptides can also discriminate among targets based on their order of connectivity. In conclusion, peptide inhibitors are able to select among heteromeric K-channel targets based on both identity of the component monomers, and on their order of connectivity. Thus, the toxins may bind across monomeric boundaries. This may account for the wide variety of selectivity "fingerprints" observed for intact cells and tissues and maybe of major relevance for the physiological action of a given peptide.

3073-Pos Board B178

Scorpion Toxins Modify C-Type Inactivation in a Mutant Potassium Channel

Azadeh Nikouee Ghadikolaei, Stephan Grissmer.

The amino acid at position 399 in the outer vestibule of *h*Kv1.3 channels (in *Shaker* 449) critically determines the C-type inactivation time course. In the present study we generated an *h*Kv1.3_H399N mutant channel with asparagines in the outer vestibule. This mutant channel showed faster inactivation and recovery time courses compared to the wild-type channel. We investigated the effect of MgTX and CTX on C-type inactivation of the mutant channel in $NMDG^+$ (K^+:4.5 mM) solutions using the whole-cell patch-clamp technique. Our results showed that the inactivation time course of the mutant channel increased around 10-fold in the presence of MgTX and 3-fold in CTX. In both cases the toxin affinity to the mutant channel is much lower compared to the wild-type channel. Other peptide toxins (NTX, AgTX2 and KTX) did not show any remarkable effects on C-type inactivation. We think that MgTX and CTX can bind to the outer vestibule of the mutant channel thereby impeding the structural changes in the outer mouth of the channel that are involved in the inactivation process. Rearrangement of the outer vestibule during C-type inactivation has been proposed earlier (Grissmer et al., 1989, *BiophysJ* **55**:203; Choi et al., 1991, *PNAS* **88**:5092; Liu et al., 1996, *Neuron* **16**:859).We conclude that C-type inactivation in voltage-gated potassium channels induce structural changes in the outer vestibule and therefore differs from the C-type inactivation in *KcsA* channels (Cuello et al., 2010, *Nature* **466**:203), which shows little changes in the outer vestibule of the *KcsA* channel.

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3074-Pos Board B179

Quantification of Non-Conducting Kv2.1 Channels in Transfected HEK Cells and Cultured Hippocampal Neurons

Philip D. Fox, Robert Loftus, Emily Deutsch, Michael M. Tamkun.

Kv2.1 potassium channels retained within cell-surface clusters in transfected HEK cells are incapable of conducting potassium. Expression of GFP-tagged Kv2.1 reveals two distinct populations of channels, those retained within clusters and those freely diffusing throughout the membrane. We hypothesized that all whole-cell current is derived from non-clustered channel. The goals of our present work were to 1) determine how the number of freely diffusing Kv2.1 channels in transfected HEK cells relates to the number of channels conducting K+, and 2) compare levels of endogenous Kv2.1 and Kv current in cultured hippocampal neurons. To quantify GFP-tagged Kv2.1

expression density in HEK cells high resolution TIRF microscopy was used to measure the intensity of individual fluorescent channels. Based on individual Kv2.1 intensity the average number of freely diffusing, non-clustered channels was ~200,000/cell whereas electrophysiological recordings of peak Kv currents corresponded to ~100,000 channels (n = 11), suggesting only 50% of the non-clustered channels are conducting K+. Endogenous Kv2.1 expression in cultured E18 hippocampal neurons at 20 days in vitro (DIV) was determined by immunocytochemistry, standardized to the GFP-Kv2.1 in HEK cells, and compared to total Kv current. Average immunofluorescence corresponded to ~60,000 channels (n = 11) in DIV 20 neurons while the ratio of clustered to non-clustered channels in DIV 20 neurons was 2:1 resulting in ~20,000 non-clustered channels. Steady-state Kv current magnitude in these neurons averaged 15 nA at +60mV (n = 4). Since 60% of this outward current is Kv2.1 mediated, there are 9,000 conducting Kv2.1 channels present. Thus, less than 50% of non-clustered Kv2.1 channels in cultured neurons conduct K+. These data also suggest that the endogenous Kv2.1 channels trapped within the cell surface clusters are held in a non-conducting state as observed in HEK cells.

3075-Pos Board B180

Effects of Electric Field on Channel Proteins Through Dipole Perturbation and Network of Signal Transmission

Gamze Gursoy, Larisa Adamian, Hsiao-Mei Lu, Jie Liang.

Kv1.2 voltage-gated and MlotiK1 cyclic nucleotide-gated K+ channels belong to the family of tetrameric cation channels and share a similar protein fold in the transmembrane region. Kv1.2 channel is activated by the changes in the transmembrane potential, while MlotiK1 channel is activated upon the binding of cyclic nucleotides to its intracellular domain. We use a perturbation-based markovian transmission model [Lu and Liang,PLoS Comp. Biol. 2009] to study allosteric activation pathways in both channels. The initial perturbation on residues, e.g., ligand binding or conformational change, is converted to flow of probability, which allows studying of the time-course of signal transmission and propagation of probability flow through the protein molecule. As dipoles in channel proteins respond to the external electric field, change in energy and introduction of torque arise for individual residues. We postulate residues that experience large energy change and torque are those responding first to the membrane depolarization in ion channels. To identify regions of initial perturbation, we build structural models by embedding channel proteins in the POPC lipid bilayer, with surrounding slabs of water molecules on both sides of the membrane. Our calculations identified S1 helix of voltage sensing domain, linker, and filter regions in Kv1.2 channel, as well as helix S1 and linker in MlotiK1 channel as the regions of initial response, as they contain the majority of strongly polarizable dipoles. Our results show that dipole perturbation results in a strong signal transmittion to the charged arginine residues of S4 in Kv1.2, whereas no significant signal transmission is observed under the same perturbation for MlotiK1 channel. This suggests dipole perturbation is a mechanism how voltage gated channel proteins respond to external electric field. This mechanism, however, is not employed by ligand-gated channels.

Voltage-gated Ca Channels

3076-Pos Board B181

Cav3.1/α1G T-Type Ca^{2+} Channels are Involved in the Heart Rate Regulation

Yingxin Li, Fang Wang, Xiaoying Zhang, Zhao Qi, Christopher Szeto, Mingxin Tang, Yinzheng Guan, Xiaojie Ai, Hongyu Zhang, Jeffery D. Molkentin, Xiongwen Chen.

T-type Ca^{2+} channels (TTCCs) are expressed in cardiac pacemaker cells and conduction system of mammals. However, the role of TTCCs in heart rate (HR) generation and regulation is not well understood. In the mouse, the major TTCC expressed in the heart is Cav3.1/α1G, and therefore we used Cav3.1/α1G transgenic (TG) and knockout (KO) mice respectively to define the role of TTCC in the heart rate generation and regulation. **Methods:** Telemetric (conscious) and surface (anesthetized) ECG were used to determine the effect of isoproterenol (ISO) on the HR *invivo*. To reduce the complication of *in vivo* HR regulation, Langendorff ECG was used to measure the response of the HR to ISO. Whole cell voltage clamp was used to measure the $I_{Ca\text{-}T}$ before and after ISO application on TG myocytes. **Results:** At baseline, telemetric ECG recording showed no significant difference in HR between the Cav3.1/α1G TG mice (536.3 ± 24.8bpm vs. FVB control: 550.6 ± 15.3bpm), Cav3.1/α1G KO mice (614.4 ± 39.9bpm vs. c57/bl6 control: 603.1 ± 64.5bpm) and control mice was detected. ISO increased the HR rate in conscious mice to the same extent in both TG (41.2 ± 6.9% vs. FVB control: 34.0 ± 3.6%) and KO (22.6 ± 8.8% vs. c57/bl6 control: 22.8 ± 8.5%) mice. However, when the central regulation is depressed (anesthetized) or removed (ex-vivo Langendorff perfusion), the percentage of HR increase after ISO application were significantly enhanced in the TG mice but reduced in KO mice. Cav3.1/α1G T-type Ca^{2+} currents ($I_{Ca\text{-}T}$) in sinoatrial nodal cells was significantly increased by 43 ± 16 % by ISO. **Conclusions:** Cav3.1/α1G TTCC might not play a major role in basal HR generation but it may play an important role in sympathetic/adrenergic regulation of HR, in which PKA could be an important mediator.

3077-Pos Board B182

Cav1.3 L-Type Calcium Channels-Mediated Ryanodine Receptor Dependent Calcium Release Controls Heart Rate

Angelo Torrente, Pietro Mesirca, Patricia Neco, Martina Sinegger-Brauns, Joerg Striessnig, Joël Nargeot, Sylvain Richard, Ana Maria Gomez, Matteo E. Mangoni.

Pacemaker activity of the sino-atrial node (SAN) controls heart rate. However, the mechanism underling SAN pacemaker activity is not completely understood and in particular, the respective physiological importance of ion channels and ryanodine receptor (RyR)-dependent Ca2+ release in pacemaking is hotly debated. We have investigated Ca2+ handling in SAN pacemaker cells of wild-type (WT) and mice lacking L-type Cav1.3 (Cav1.3-/-) channels. In isolated Cav1.3-/- SAN cells the frequency of Ca2+ transients was reduced by 45% compared to WT pacemaker cells. Loss of Cav1.3 channels also blunted by about 47% the positive chronotropic effect induced by 0.01 μM isoproterenol (ISO). Furthermore, in Cav1.3-/- pacemaker cells, local Ca2+ release (LCR) occurring during the diastolic phase was reduced by 71%. In SAN cells from mice in which L-type Cav1.2 channels have been rendered insensitive to dihydropyridines (Cav1.2DHP-/-), application of 0.3 μM isradipine decreased diastolic LCR by 78 %, thus showing that Cav1.3 channels are major regulators of RyR-dependent LCR during the diastolic phase. In individual cells of isolated intact SAN, pacemaking of Cav1.3-/- cells was characterized by reduced (37%) frequency of Ca2+ transients and an increase in Ca2+ waves. Normal pacemaking in Cav1.3-/- isolated SAN cells and intact tissue could be observed only after direct activation of RyR-dependent Ca2+ release by low doses of caffeine (200 μM). Experiments with high doses of caffeine (10 mM) in Cav1.3-/- cells, showed that the reduction in diastolic LCR and in the frequency Ca2+ transients could not be ascribed to a decrease in sarcoplasmic reticulum (SR) Ca2+ content. Our results show that in SAN pacemaker cell, LCR Ca2+ release is tightly controlled by Cav1.3 channels and that such a control is critical for promoting the formation of whole-cell Ca2+ transients.

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3078-Pos Board B183

Evidence for a Role for the Cytoskeleton in Communication Between the L-Type Calcium Channel and the Mitochondria in Isolated Cardiac Myocytes

Helena M. Viola, Livia C. Hool.

Cytoskeletal proteins stabilize cell structure but also regulate subcellular distribution of mitochondria and cardiac L-type Ca^{2+} channel (LTCC) activity. We have previously demonstrated that mitochondrial function can be regulated by alterations in LTCC activity. This effect was attenuated when the cytoskeleton was disrupted with latrunculin A. To further explore this, we determined whether regulation of mitochondrial function by the LTCC is altered in a murine model of Duchenne Muscular Dystrophy(*mdx*). Mitochondrial membrane potential (Ψ_m) and metabolic activity was assessed after activation of the LTCC in cardiac myocytes isolated from C57BL/10ScSn-Dmdmdx/Arc (*mdx*) and C57BL/10ScSnArc (control) mice. Exposure of control myocytes to 10μM BayK(-) (LTCC agonist) caused a 11.4 ± 1.7% increase in Ψ_m assessed as alterations in JC-1 compared to myocytes exposed to inactive BayK(+) (n=8, $p<0.05$). The response was attenuated when myocytes were exposed to LTCC antagonist nisoldipine (n=7). However BayK(-) did not induce any significant alteration in JC-1 signal in myocytes from *mdx* mice (n=6). In control myocytes BayK(-) caused a 105.4 ± 7.4% increase in metabolic activity assessed using an MTT assay (n=8, $p<0.05$). The response was attenuated when myocytes were exposed to nisoldipine (n=8) or mitochondrial calcium uniporter inhibitor Ru360 (n=8) but unaltered when exposed to ryanodine receptor antagonist dantrolene (n=4). Exposure of *mdx* myocytes to BayK(-) did not induce any significant alteration in metabolic activity (n=8). These data confirm that alterations in LTCC activity can modulate mitochondrial function and that the cytoskeleton plays an important role in mediating this response. Since the LTCC is the initiator of contraction it has been proposed that a functional coupling between the LTCC and mitochondria may assist in meeting myocardial energy demand on a beat to beat basis.